Environment International 145 (2020) 106095

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Pre- and post-diagnostic blood profiles of perfluoroalkyl acids in type 2 diabetes mellitus cases and controls

Dolley Charles[a,*], Vivian Berg[b,c], Therese H. Nøst[a], Sandra Huber[c], Torkjel M. Sandanger[a], Charlotta Rylander[a]

[a] Department of Community Medicine, Faculty of Health Sciences, UIT-The Arctic University of Norway, NO-9037 Tromsø, Norway
[b] Department of Medical Biology, Faculty of Health Sciences, UIT-The Arctic University of Norway, NO-9037 Tromsø, Norway
[c] Department of Laboratory Medicine, Division of Diagnostic Services, University Hospital of North-Norway, NO-9038 Tromsø, Norway

ARTICLE INFO

Handling Editor: Olga-Ioanna Kalantzi

Keywords:
Perfluoroalkyl substances
Type 2 diabetes mellitus
Pre- and post-diagnostic associations
Longitudinal assessment
Perfluoroalkyl acids
Repeated measurements

ABSTRACT

Background: Studies exploring the associations between perfluoroalkyl acids (PFAAs) and type 2 diabetes mellitus (T2DM) are rather limited and have reported conflicting results. All studies to date, including prospective ones, have relied on a single blood sample to study this association. Similarly, studies investigating how T2DM status may influence the longitudinal changes in PFAA concentrations have not been previously performed. As PFAA concentrations in humans have changed considerably over the last two decades, and as individuals diagnosed with T2DM usually undergo lifestyle changes that could influence these concentrations, a single blood sample may not necessarily reflect the life-time exposure to PFAA concentrations. Hence, repeated measurements from the same individuals will extend our understanding of how PFAAs are associated with T2DM. The present study, therefore, aimed to explore associations between pre- and post-diagnostic PFAA blood profiles and T2DM and assess factors associated with longitudinal changes in PFAAs in T2DM cases and controls.

Methods: Questionnaire data and blood samples from women participating in the Norwegian Women and Cancer study were used to conduct a nested case-control study among 46 T2DM cases matched to 85 non-diabetic controls. PFAAs were measured in blood samples collected prior to (2001/02) and after (2005/6) T2DM diagnosis. We investigated the association between PFAAs and incident and prevalent T2DM using conditional logistic regression. We assessed the longitudinal changes in PFAA concentrations within and between matched cases and controls using t-tests and linear regression models.

Results: We observed no significant associations between pre-diagnostic PFAA concentrations and T2DM incidence. Similar results were observed for the post-diagnostic PFAA concentrations and T2DM prevalence. Decrease over time in PFAA concentrations were observed for PFOA and ΣPFOS concentrations, whereas increase over time were observed for PFNA, PFDA and PFUnDA concentrations. Longitudinal trends in PFAA concentrations among T2DM cases were similar to the changes observed in controls.

Conclusions: The study did not find evidence of association between PFAAs and incident or prevalent T2DM. The longitudinal changes in PFAAs concentrations were not influenced by T2DM status.

1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM), a metabolic condition characterized by elevated blood glucose levels, has increased alarmingly worldwide and accounts for 90% of all diabetes (Saeedi et al., 2019). The estimated global prevalence of diabetes was 9.3% (463 million people) in 2019, and projections suggest that it will rise to 10.2% by 2030 and 10.9% by 2045 (Saeedi et al., 2019). Well-established risk factors of T2DM include older age, obesity, sedentary lifestyle, and genetic predisposition. Diet is considered a risk factor for T2DM, however, previous studies have shown that dietary factors associated with increased risk for T2DM are linked with other unhealthy lifestyle factors which showed highly significant associations with T2DM, such as physical inactivity and increased BMI (Aune et al., 2009; Bellou et al., 2018; Imamura et al., 2015). However, recent research has implied that other non-traditional factors like stress, epigenetic changes, and various environmental organic pollutants may also contribute to the increased prevalence of T2DM (Magliano et al., 2014; McAllister et al., 2009).

Legacy persistent organic pollutants, such as polychlorinated

* Corresponding author.
E-mail address: dolley.charles@uit.no (D. Charles).

https://doi.org/10.1016/j.envint.2020.106095
Received 9 May 2020; Received in revised form 24 August 2020; Accepted 25 August 2020
Available online 09 September 2020

biphenyls (PCBs) and organochlorine pesticides (OCPs), are endocrine disrupting chemicals that may play a role in the development of metabolic conditions like T2DM (Alonso-Magdalena et al., 2011; Nadal et al., 2017; Taylor et al., 2013). However, evidence for emerging pollutants like perfluoroalkyl substances (PFASs) is rather limited.

PFASs are a class of organofluorine compounds that have been widely used in industrial and consumer products since the 1950s. Many PFASs are persistent and accumulate in the environment and in biota; today, the main route of human exposure to PFASs is through diet (Lin Pi et al., 2020; Vestergren et al., 2008; Vestergren and Cousins, 2009). Other sources of exposure include PFAS-treated clothing, food packaging materials, and cooking utensils, but also dust inhalation and skin absorption (Haug et al., 2011; Lau, 2015; Nadal et al., 2017). The most frequently detected of such compounds in human blood are perfluoroalkyl acids (PFAAs), a subclass of the PFAS family, in which perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the most frequently studied. The elimination half-lives of PFAAs were estimated to be 3.5–8.5 years (Olsen et al., 2007a).

PFAAs are classified as endocrine disrupting chemicals (Lind and Lind, 2018). They have a chemical structure that resembles that of fatty acids, a feature that enables them to bind to and activate peroxisome proliferator-activated receptors. These receptors play an important role in lipid and glucose metabolism and in the regulation of energy homeostasis (Jiang et al., 2015; Lind et al., 2014; Wolf et al., 2008). Therefore, pharmaceutical drugs target these receptors for the treatment of dyslipidemia and T2DM. Since PFAAs bind to the same receptors, the effects that PFAAs may have on glucose metabolism are worth exploring (Lind et al., 2014). Other proposed mechanisms include effects of PFAAs on thyroid and steroid hormones which play a major role in adipocyte differentiation and energy storage which in turn may increase the risk of T2DM (Hatch et al., 2010). Animal studies have established no direct link between PFAA exposure and the pathogenesis of T2DM (Khalil et al., 2015). However, findings from both cross-sectional and prospective studies of PFAA concentrations in the blood of humans have reported inconsistent results: some studies have reported null (Cardenas et al., 2017; Karnes et al., 2014; Lind et al., 2014) or inverse associations (Donat-Vargas et al., 2019; MacNeil et al., 2009), and a few studies have reported positive associations (Christensen et al., 2016; He et al., 2018; Sun et al., 2018). Thus, the role of circulating PFAA concentrations in the development of T2DM is uncertain and needs to be further investigated with prospective studies. In addition, all studies to date have reported results from a single blood sample collected either prior or after T2DM diagnosis. As the general Norwegian population experienced a considerable increase in PFAA concentrations until the year 2001 (Nøst et al., 2014), followed by a significant decline, a single blood sample may not reflect lifetime exposure to PFAAs. Further, after T2DM diagnosis, many patients adopt lifestyle changes and are prescribed glucose-lowering drugs; both of these factors may affect the concentration of fat-soluble PCBs and OCPs (Tornevi et al., 2019). Little is known about whether and how these factors influence PFAA concentrations in T2DM patients. Therefore, the present study aimed to explore associations between pre- and post-diagnostic PFAA blood profiles and T2DM among T2DM cases and controls and explore the factors influencing the longitudinal trends in PFAAs concentrations using a longitudinal, nested case-control design.

2. Materials and methods

2.1. The Norwegian Women and Cancer study

The Norwegian Woman and Cancer (NOWAC) study, established in 1991, is an ongoing, population-based, prospective cohort study. The cohort is nationally representative and consists of over 170,000 women (30–70 years of age) who have answered between one and four extensive questionnaires on diet, lifestyle factors, medications, and self-reported diseases (Lund et al., 2008). Approximately, 50,000 participants have also given blood samples, which are stored at −80 °C, and completed a questionnaire about their use of medications at the time of blood collection. A total of 7849 women donated blood samples at two separate time points: time point 1 (T1) in 2001/02 and time point 2 (T2) in 2005/06. A detailed description of blood collection procedures has been reported elsewhere (Waaseth et al., 2008).

2.2. Study design and participants

This is a longitudinal, 1:2 individually-matched, nested case-control study. T2DM cases were defined as those reporting diabetes in the NOWAC questionnaire and/or reporting the use of diabetes medication at T2. This questionnaire information has been previously validated against medical journals/doctors confirmation in the NOWAC study (Rylander et al., 2014). Of the 7849 women who provided blood samples at T1 and T2, 53 were free of T2DM at T1 and reported T2DM at T2. Blood samples collected at T1 were then defined as pre-diagnostic samples (among cases) and those taken at T2 as post-diagnostic samples. Women with cancer, and those with an insufficient amount of plasma available were excluded, as were women with diabetes who were taking insulin (to ensure that no type 1 diabetes cases were included), leaving 46 T2DM cases in the analytical sample. Each T2DM case was then matched with two diabetes-free controls; control 1 was matched on birth year (± 1 year) at T1 and year of blood collection at T2; control 2 was matched on birth year (± 1 year) at T1, body mass index (BMI) (± 3 kg/m^2) at T2, and year of blood collection at T1 and T2. Our study is part of a larger study that intended to explore the relationship between lipophilic PCBs, OCPs, and hydrophilic PFAAs and T2DM. Since evidence shows that BMI is directly linked with both PCBs, OCP concentrations and T2DM (a confounder), we matched control group 2 on BMI at T2. Due to lack of sufficient plasma volume, two controls from control group 1 were excluded. In control group 2, only 41 available controls could be matched on both birth year and BMI. Thus, case-control group 1 consisted of 44 matched pairs and case-control group 2 consisted of 41 matched pairs.

2.3. Questionnaire data

Information on covariates was retrieved from NOWAC questionnaires. Each participant answered five questionnaires (Qs), in 1991 (Q1), 1998 (Q2), 2001/02 (Q3), 2004/05 (Q4), and 2005/06 (Q6). These questionnaires included detailed information on demographics, lifestyle factors, dietary factors, anthropometrics, health related questions, use of medications, and information on parity and total months of breastfeeding. Age, weight, and height were reported in all questionnaires except Q3 (filled out at T1). Information on breastfeeding and parity was only reported in Q1 and Q2.

2.4. Chemical analysis

A total of 18 PFAAs were analyzed at the Department of Laboratory Medicine, University Hospital of North Norway (Table A.1). The procedures for sample preparation, instrumental analysis, quantification, and quality control for PFASs have been previously described in detail (Huber and Brox, 2015). In short, an automated liquid handler (Tecan Freedom Evo 200, Männedorf, Switzerland) was used for the preparation of extracts, where 50 μL of plasma was applied. Instrumental analysis was conducted on a Waters Acquity ultra-high-pressure liquid chromatography system coupled to a Waters Xevo-TQ-S tandem mass-spectrometer (both Waters, Milford, MA, USA). Electrospray ionization in negative mode was applied for ionization of the analytes and multi-reaction monitoring mode for recording of the transitions. For quality assurance, four blank samples, four standard reference material (SRM) 1958 and SRM 1957 (NIST, Gaithersburg, MD, USA), and three bovine serum samples (Sigma Aldrich, Steinheim, Germany) were prepared and analyzed within each batch of 96 samples in order to control

background and carry-over. All the quality controls were within acceptable limits (within three times the standard deviation from the reference concentrations, together with a relative standard deviation of ≤15%), and all PFAA analyses were within the acceptable ranges (z-score of ≤1) of the international quality control program: the Arctic Monitoring and Assessment Ring Test for Persistent Organic Pollutants in Human Serum (organized by the Laboratoire de toxicologie, Institut National de Santé Publique du Quebec, Canada).

2.5. Statistical analysis

All statistical analyses were performed using STATA software, version 16 (StataCorp, 4905 Lakeway Drive, College Station, TX, USA). Only those PFAAs that had a detection frequency ≥90% were included in the statistical analyses. These included seven PFAA compounds – four perfluoroalkyl carboxylic acids: PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA); and three perfluoroalkyl sulfonic acids: perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), and PFOS. PFAA concentrations below their individual method detection limits (MDL) were replaced by MDL divided by 2 (Table A.1). The linear and branched forms of PFHxS, PFHpS, and PFOS were summed and presented as ΣPFHxS, ΣPFHpS, and ΣPFOS, respectively. The individual PFAA compounds were ranked in the order of lowest to highest. The sum of the ranks of PFNA, PFOA, PFDA, PFUnDA, ΣPFHxS, ΣPFHpS, and ΣPFOS concentrations is presented as ΣPFAAs. Spearman correlations were performed to examine the linear relationship between different PFAAs at the two different time-points.

Descriptive statistics at T1 are presented as mean and standard deviation (SD) for demographic data and median (5th, 95th percentiles) for PFAA concentrations (ng/mL). We compared demographics and PFAA concentrations between cases and matched controls at T1 and T2 (mean in cases-mean in controls) using one-sample t-tests. We also tested whether the longitudinal changes (δ) within individuals from T1 to T2 were significantly different from zero (T2 measurement – T1 measurement) using paired t-tests. Comparison of trends between cases and controls (longitudinal changes in cases – longitudinal changes in controls) were analyzed using one-sample t-tests. Thus, we tested the null hypothesis that the longitudinal change in cases equaled the longitudinal change in controls. In order to control for confounding factors and identify factors associated with the longitudinal changes in PFAAs, we performed linear regression models using the longitudinal changes in PFAA concentration from T1 to T2 as dependent variables. Age at T1 centered around the mean age of 52, BMI at T1 centered around BMI = 25 kg/m^2, dietary changes (fish, meat, dairy, fruits and vegetables) and weight change from T1 to T2 were included as independent variables. We did not consider changes in parity and breastfeeding in the linear regression models as the participants were over 50 years of age at T1. BMI at T1 and weight change from T1 to T2 served as proxies for the matching on BMI at T2 for case-control group 2.

The association between pre-diagnostic PFAA concentrations and odds of T2DM at T1 were examined using multivariable conditional logistic regression. The covariates considered were age, breastfeeding and dietary factors (meat, fish, diary, fruit and vegetables). The selection of covariates was based on previous literature and drawing a directed acyclic graph (DAG) showing the assumed relations between PFAAs, T2DM, and the different covariates (Fig. 1). Weight and height at T1 were extracted from Q2. Since breastfeeding and parity were found to be highly correlated, only breastfeeding was considered for the logistic regression models. The covariates identified from the DAG was also included in the conditional logistic regression models exploring the associations between the post-diagnostic PFAA concentrations and prevalent T2DM. The results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). ORs are estimated per 1 interquartile range (IQR) increase in PFAA concentrations and 50 ranks increase in ΣPFAAs. All p-values were two-sided, and a 5% level of significance was used.

3. Results

3.1. Study sample characteristics

At T1 (pre-diagnosis), the mean age and BMI for the whole study sample were 52.0 years and 26.9 kg/m^2, respectively. Cases had significantly higher weight than both control groups and higher meat intake compared to control group 2. However, there were no significant differences in parity, breastfeeding or the other dietary factors. At T2, only BMI was significantly higher in cases compared to control group 1. No differences in demographic variables or dietary factors were observed between cases and control group 2 (Table 1).

At both time points, ΣPFOS and PFOA were the two most prevalent PFAAs measured in both cases and controls. No significant differences were observed in mean PFOA, PFNA, PFDA, PFUnDA, ΣPFHxS, ΣPFHpS and ΣPFOS concentrations between cases and control groups at T1. At T2, cases had significantly lower PFOA concentrations compared to control group 2, whereas there were no differences across case-control pairs for any of the other PFAAs at T2 (Table 1). Strong positive correlations were observed between the different PFAAs at both the time-points with the correlation coefficient (r_s) ranging between 0.52–0.92 at T1, and 0.54–0.90 at T2 (Table A.2).

3.2. Longitudinal changes within cases and controls

Within cases, there were no significant changes in weight, BMI, parity, or breastfeeding from T1 to T2. Cases showed no mean difference in dietary factors from T1 to T2 (Table A.3). PFOA and ΣPFOS concentrations significantly decreased, whereas PFNA, PFDA, PFUnDA, and ΣPFHxS concentrations increased significantly from T1 to T2. ΣPFHpS showed no significant changes (Fig. 2, Table A.3). Both control groups increased significantly in weight, BMI, fruits and vegetables intake from T1 to T2, but there were no significant changes in parity, breastfeeding or fish intake. Reduced dairy intake in control group 1 and increased meat intake in control group 2 were observed at T2 (Table A.3). ΣPFOS decreased significantly in both control groups, and there were no significant changes in PFOA, ΣPFHxS, or ΣPFHpS concentrations. PFNA, PFDA, and PFUnDA concentrations increased significantly from T1 to T2 in both control groups (Fig. 2, Table A.3)

3.3. Longitudinal changes between cases and controls

Mean change in weight and BMI between T1 and T2 were lower for cases compared to both control groups; however, this change was only statistically significant for the comparison of BMI between cases and control group 2. The crude longitudinal changes in PFAA concentrations from T1 to T2 did not differ significantly between cases and control groups 1 or 2, except for ΣPFHpS, for which a significantly larger decline in concentration over time was observed among cases compared to both control groups (Fig. 2, Table A.4). However, after controlling for age and BMI at T1, changes in diet and body weight, T2DM status did not seem to influence the longitudinal trends in any of the PFAA compounds, but changes in PFAAs over time were rather driven by age and dietary factors (Table A.5).

3.4. Pre-diagnostic and post-diagnostic associations

After adjusting for relevant confounders, none of the PFAAs were significantly associated with increased or decreased odds of T2DM when measured at T1 (pre-diagnosis) or at T2 (post-diagnosis) (Tables 2 and 3). Inverse associations for pre-diagnostic concentrations of PFOA and ΣPFHpS were observed for both case control groups. PFNA, PFDA, PFUnDA, ΣPFHpS, ΣPFOS, and ΣPFAAs concentrations showed inverse

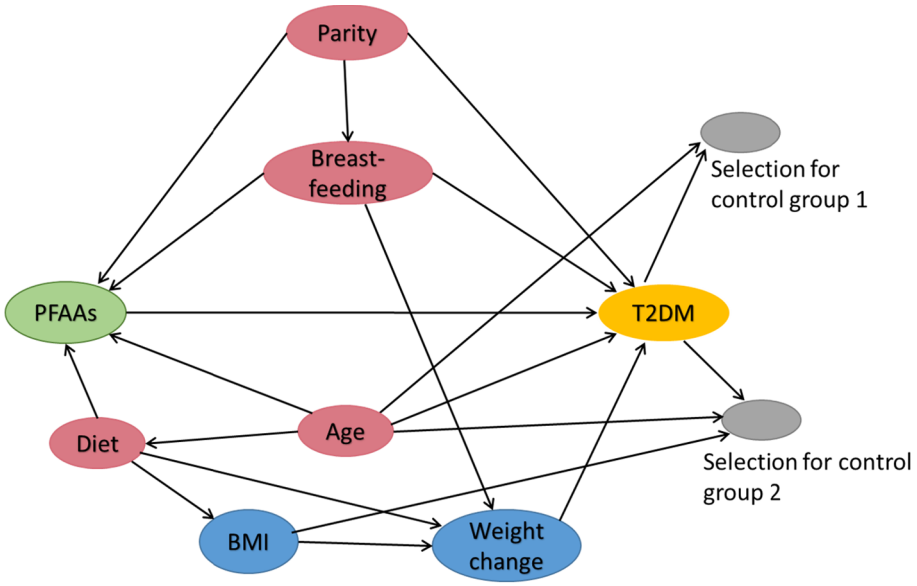

Fig. 1. Directed acyclic graph of the causal network between pre-diagnostic PFAAs and risk of type 2 diabetes mellitus. (The different colors in the DAG represent the following: green-exposure; yellow-outcome; pink-confounders; blue-mediators; grey-matching factors for control groups; arrows-direction of the pathways). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

associations for case-control group 1 and positive associations for case control group 2. However, none of the pre-diagnostic associations were statistically significant in the multivariable adjusted models for either of the case control groups. (Tables 2 and 3). Inverse and non-significant associations were observed between prevalent T2DM and post-diagnostic PFOA, PFNA, ΣPFHxS, ΣPFHpS, ΣPFOS, and ΣPFAA concentrations, and positive, non-significant associations for PFDA and PFUnDA in the multivariable adjusted models for control group 2 (Tables 2 and 3).

4. Discussion

In this study, none of the investigated PFAAs were significantly associated with increased odds of T2DM when measured prior to or after disease development. There were significant changes in PFAA concentrations from pre- to post-diagnosis within cases and controls; ΣPFOS concentrations decreased and PFNA, PFDA, and PFUnDA concentrations increased within cases and both control-groups, whereas PFOA concentrations decreased and ΣPFHxS increased significantly within cases. However, the longitudinal changes were not significantly different in cases versus controls and were mainly explained by differences in age and dietary intake after controlling for confounding factors. Thus, T2DM status did not influence the longitudinal changes in PFAAs.

Overall, our study results of no associations between pre-diagnostic PFAA concentrations and incident T2DM are in line with the findings from other prospective studies. For instance, the most recent, prospective, nested case-control study by Donat-Vargas et al. (2019) found inverse, mostly non-significant associations between pre-diagnostic PFAA concentrations (PFOA, PFDA, PFUnDA, PFHxS, and PFOS) and odds of T2DM in 124 case-controls pairs from the Swedish, prospective, population-based Västerbotten Intervention Programme cohort (Donat-Vargas et al., 2019). Another large prospective study conducted among residents of a community exposed to high levels of PFOA through drinking water (C8 Health project) presented a null association between PFOA and incident T2DM (n = 27,921; 814 cases). However, in that study, serum PFAA concentrations were only estimated and not measured (Karnes et al., 2014). A study conducted among overweight and obese individuals at high risk for T2DM (n = 957,204; T2DM cases) found no association between pre-diagnostic PFAA concentrations (PFNA, PFOA, ΣPFHxS, and ΣPFOS) and incident T2DM (Cardenas et al., 2017). In contrast to our results, positive, significant associations between pre-diagnostic PFOA concentrations (3rd vs. 1st tertile OR: 1.54, 95% CI: 1.04, 2.28), PFOS concentrations (3rd vs. 1st tertile OR: 1.62, 95% CI: 1.09, 2.41), and incident T2DM were reported by a recent, prospective, nested case-control study (n = 1586, 793 T2DM cases) conducted among women from the United States Nurses Health Study II. No association was observed between T2DM and PFNA, PFDA, or ΣPFHxS in that study. However, they reported PFAA concentrations (median PFOA and ΣPFOS concentrations of 7.36 ng/mL and 56.3 ng/mL, respectively) that were much higher than those we report in our study. Only the tertiles with the lowest concentrations (2.89 ng/mL for PFOA and 19.7 ng/ml for PFOS) were comparable to the concentrations in our sample (Sun et al., 2018). The different prospective studies vary in sample size, type of study participants (difference in gender, high-risk individuals, and general population or highly exposed individuals), median PFAA concentrations, year(s) of blood sampling, number of follow-up years, and statistical analyses. These differences make it difficult to extrapolate our findings to other prospective studies. For instance, evidence for increased risk of T2DM in general populations with high PFAA concentrations in the blood is still unclear. Thus, the dose–response relationship between different PFAA exposure concentrations and T2DM incidence in the general population needs further attention.

Similar to T1, at T2 (post-diagnosis), PFAA concentrations showed no associations with T2DM prevalence. In agreement with our results, previous studies that have examined this association also reported no associations between PFAAs and T2DM prevalence (He et al., 2018; Lind et al., 2014; MacNeil et al., 2009; Rylander et al., 2015). For instance, He et al. (2018) conducted a large, cross-sectional study of 7904 adults in the National Health and Nutrition Examination Survey in the United States. PFOA, PFNA, and PFHxS concentrations were slightly higher and PFOS was slightly lower in those samples compared to our study. They reported no association between serum PFOA and prevalent T2DM among women (n = 3948, OR: 1.47, 95% CI: 0.88, 2.46; P for trend = 0.737). However, a significant positive association was reported among men in the same study (n = 3956, OR: 2.66, 95% CI: 1.63, 4.35; P for trend = 0.001). The other PFAAs studied (PFNA, PFHxS, and PFOS) were not related to diabetes, regardless of gender (He et al., 2018). Other studies have also reported significant positive associations between certain PFAAs and prevalent T2DM in men (Christensen et al., 2016). Our study results are in agreement with previous studies that found no association between PFAAs and T2DM prevalence in women. The fact that other studies have observed significant associations in men indicates that there could be gender-dependent changes in PFAA concentrations after T2DM diagnosis.

Table 1
Demographic characteristics, dietary factors and PFAA concentrations of cases and controls and mean difference (Δ) between case-control pairs.

		T1 (pre-diagnosis)			T2 (post-diagnosis)		
		Mean (SD)	Median (5, 95 percentiles)	$\Delta Mean_{case\text{-}control}$ (95% CI)[e]	Mean (SD)	Median (5, 95 percentiles)	$\Delta Mean_{case\text{-}control}$ (95% CI)[e]
Case-control group 1							
Demographics							
Age (years)	Cases[a]	52.2 (4.93)	55.0 (45.0, 58.0)	0.25	56.1 (4.84)	58.5 (49.0, 61.0)	0.20
	Controls 1[b]	51.9 (4.92)	54.0 (44.0, 58.0)	(0.04, 0.46)	56.0 (4.90)	58.0 (48.0, 62.0)	(−0.02, 0.43)
Weight (kg)	Cases[a]	78.1 (16.8)	75.0 (60.0, 120)	6.30	79.8 (17.1)	77.0 (59.0, 110)	5.34
	Controls 1[b]	71.8 (14.2)	70.0 (53.0, 100)	(0.14, 12.4)	74.4 (15.9)	74.0 (55.0, 112)	(−0.82, 11.5)
BMI (kg/m^2)	Cases[a]	28.5 (5.50)	27.6 (21.0, 39.6)	2.67	29.2 (5.85)	28.3 (21.1, 40.6)	2.38
	Controls 1[b]	25.9 (4.52)	24.5 (20.4, 35.4)	(0.64, 4.70)	26.8 (5.04)	25.6 (20.7, 39.8)	(0.41, 4.36)
Parity	Cases[a]	2.32 (1.25)	2.00 (0.00, 4.00)	0.18	2.34 (1.24)	2.00 (0.00, 4.00)	0.18
	Controls 1[b]	2.14 (1.13)	2.00 (0.00, 4.00)	(−0.34, 0.71)	2.16 (1.11)	2.00 (0.00, 4.00)	(−0.33, 0.70)
Breastfeeding (months)	Cases[a]	12.3 (11.6)	10.0 (1.00, 36.0)	3.69	12.3 (11.6)	10.0 (1.00, 36.0)	3.69
	Controls 1[b]	8.65 (8.31)	7.00 (0.00, 24.0)	(−1.10, 8.46)	8.65 (8.31)	7.00 (0.00, 24.0)	(−4.83, 5.22)
Fish intake (g/day)	Cases	92 (54.2)	84 (17.3, 193)	−2.71	93 (47.2)	89 (33.4, 178)	−2.78
	Controls 1[b]	94 (50.8)	88 (24.4, 170)	(−21.6, 16.2)	95 (62.2)	75 (20.6, 228)	(−26.4, 20.9)
Meat intake (g/day)	Cases	122 (41.5)	126 (52.1, 177)	7.72	121 (32.7)	117 (71.9, 171)	17.3
	Controls 1[b]	114 (51.0)	104 (45.9, 202)	(−12.1, 27.5)	103 (49.2)	103 (40.1, 199)	(−1.22, 35.9)
Dairy intake (g/day)	Cases	266 (206)	240 (41.1, 674)	−21.8	230 (200)	228 (23.6, 578)	3.05
	Controls 1[b]	296 (223)	224 (30.4, 675)	(−111, 67.7)	232 (207)	154 (30.9, 609)	(−69.2, 75.3)
Fruits and vegetables intake (g/day)	Cases	360 (242)	298 (67.5, 895)	43.3	430 (362)	417 (130, 743)	30.7
	Controls 1[b]	322 (150)	286 (130, 614)	(−50.5, 137)	390 (176)	401 (150, 695)	(−62.1, 123)
PFAA compounds (ng/mL)							
PFOA	Cases[a]	2.52 (1.20)	2.32 (1.00, 4.13)	−0.27	2.35 (1.25)	2.10 (0.75, 4.54)	−0.30
	Controls 1[b]	2.79 (1.38)	2.41 (1.19, 4.90)	(−0.75 0.21)	2.66 (1.26)	2.52 (1.20, 5.59)	(−0.83, 0.22)
PFNA	Cases[a]	0.46 (0.25)	0.38 (0.18, 1.06)	−0.03	0.66 (0.37)	0.55 (0.26, 1.18)	−0.02
	Controls 1[b]	0.50 (0.25)	0.48 (0.27, 1.12)	(−0.12, 0.05)	0.69 (0.35)	0.60 (0.33, 1.45)	(−0.15, 0.11)
PFDA	Cases[a]	0.23 (0.14)	0.18 (0.07, 0.55)	−0.01	0.30 (0.18)	0.25 (0.12, 0.70)	−0.02
	Controls 1[b]	0.24 (0.14)	0.23 (0.12, 0.45)	(−0.06, 0.04)	0.33 (0.19)	0.28 (0.15, 0.72)	(−0.09, 0.04)
PFUnDA	Cases[a]	0.28 (0.17)	0.22 (0.11, 0.63)	−0.03	0.38 (0.27)	0.30 (0.12, 1.07)	−0.02
	Controls 1[b]	0.31 (0.21)	0.28 (0.15, 0.58)	(−0.10, 0.03)	0.40 (0.28)	0.33 (0.17, 1.10)	(−0.11, 0.06)
ΣPFHxS	Cases[a]	0.99 (0.64)	0.80 (0.36, 2.51)	−0.31	1.13 (0.77)	0.93 (0.33, 3.05)	−0.18
	Controls 1[b]	1.30 (1.31)	0.86 (0.39, 4.29)	(−0.75, 0.13)	1.30 (1.08)	1.05 (0.43, 3.85)	(−0.61, 0.26)
ΣPFHpS	Cases[a]	0.35 (0.17)	0.33 (0.15, 0.61)	−0.04	0.33 (0.18)	0.30 (0.12, 0.58)	−0.04
	Controls 1[b]	0.38 (0.33)	0.29 (0.13, 1.03)	(−0.14, 0.06)	0.37 (0.29)	0.29 (0.11, 0.96)	(−0.13, 0.06)
ΣPFOS	Cases[a]	22.2 (9.95)	20.1 (10.3, 37.9)	−2.99	18.5 (9.83)	16.0 (6.54, 32.0)	−3.20
	Controls 1[b]	25.2 (23.0)	19.0 (10.6, 70.2)	(−10.3, 4.36)	21.7 (20.0)	16.0 (6.09, 65.4)	(−9.69, 3.29)
Case-control group 2							
Demographics							
Age (years)	Cases[c]	52.0 (5.02)	55.0 (45.0, 58.0)	0.02	55.9 (4.89)	58.0 (49.0, 61.0)	−0.05
	Controls 2[d]	52.0 (4.42)	53.0 (46.0, 57.0)	(−0.51, 0.55)	56.0 (4.54)	57.0 (49.0, 61.0)	(−0.47, 0.37)
Weight (kg)	Cases[c]	77.3 (16.1)	75.0 (60.0, 105)	6.44	77.1 (15.9)	75.0 (58.0, 106)	2.63
	Controls 2[d]	70.9 (10.4)	70.0 (56.0, 85.0)	(1.99, 10.9)	74.4 (10.4)	74.0 (60.0, 92.0)	(−0.98, 6.25)
BMI (kg/m^2)	Cases[c]	28.2 (5.30)	27.6 (21.0, 36.6)	1.88	28.1 (5.33)	28.2 (20.7, 36.5)	0.47
	Controls 2[d]	26.3 (4.22)	25.3 (21.1, 31.8)	(0.59, 3.17)	27.7 (4.38)	26.0 (22.6, 36.7)	(−0.44, 1.38)
Parity	Cases[c]	2.15 (1.15)	2.00 (0.00, 4.00)	−0.22	2.17 (1.14)	2.00 (0.00, 4.00)	−0.20
	Controls 2[d]	2.37 (0.89)	2.00 (1.00, 3.00)	(−0.65, 0.21)	2.37 (0.89)	2.00 (1.00, 3.00)	(−0.63, 0.24)
Breastfeeding (months)	Cases[c]	12.0 (12.0)	8.00 (0.00, 36.0)	0.19	12.0 (12.0)	8.00 (0.00, 36.0)	0.19
	Controls 2[d]	12.3 (12.3)	9.00 (0.00, 30.5)	(−4.83, 5.22)	12.3 (12.3)	9.00 (0.00, 30.5)	(−4.83, 5.22)
Fish intake (g/day)	Cases	89 (51.0)	84 (17.3, 173)	−5.37	94 (48.8)	89 (33.4, 178)	−0.17
	Controls 2[d]	94 (48.4)	91 (32.2, 163)	(−28.2, 17.5)	94 (63.8)	73 (27.7, 224)	(−23.4, 23.1)
Meat intake (g/day)	Cases	123 (41.0)	124 (60.4, 177)	24.9	123 (33.1)	119 (71.9, 171)	4.83
	Controls 2[d]	98.0 (50.3)	87 (25.9, 165)	(2.09, 47.7)	118 (44.4)	115 (46.1, 190)	(−12.1, 21.8)
Dairy intake (g/day)	Cases	264 (208)	228 (41.1, 674)	−0.29	234 (210)	209 (23.6, 578)	19.8
	Controls 2[d]	264 (210)	222 (35.0, 608)	(−93.8, 93.2)	214 (181)	170 (33.9, 598)	(−71.3, 111)
Fruits and vegetables intake (g/day)	Cases	372 (245)	307 (67.5, 895)	68.3	444 (229)	422 (130, 826)	6.79
	Controls 2[d]	304 (132)	284 (122, 529)	(−15.8, 152)	437 (169)	384 (251, 695)	(−58.0, 71.6)
PFAA compounds (ng/mL)							
PFOA	Cases[c]	2.52 (1.22)	2.32 (0.99, 4.13)	−0.59	2.34 (1.24)	2.12 (0.75, 4.54)	−0.63
	Controls 2[d]	3.12 (1.69)	2.88 (1.30, 5.75)	(−1.21, 0.03)	2.98 (1.72)	2.64 (1.32, 7.10)	(−1.20, −0.07)
PFNA	Cases[c]	0.46 (0.26)	0.38 (0.18, 1.06)	−0.01	0.65 (0.37)	0.54 (0.26, 1.18)	−0.01
	Controls 2[d]	0.47 (0.23)	0.46 (0.18, 0.92)	(−0.12, 0.09)	0.66 (0.32)	0.61 (0.28, 1.23)	(−0.16, 0.13)
PFDA	Cases[c]	0.23 (0.14)	0.18 (0.07, 0.55)	0.001	0.31 (0.19)	0.25 (0.12, 0.70)	0.005
	Controls 2[d]	0.23 (0.10)	0.23 (0.10, 0.43)	(−0.05, 0.05)	0.30 (0.13)	0.28 (0.12, 0.61)	(−0.07, 0.08)
PFUnDA	Cases[c]	0.29 (0.18)	0.24 (0.11, 0.63)	−0.007	0.40 (0.28)	0.34 (0.13, 1.07)	0.04
	Controls 2[d]	0.30 (0.13)	0.30 (0.09, 0.55)	(−0.07, 0.06)	0.36 (0.17)	0.33 (0.09, 0.62)	(−0.06, 0.14)
ΣPFHxS	Cases[c]	1.03 (0.65)	0.91 (0.36, 2.51)	−0.30	1.15 (0.78)	0.95 (0.33, 3.05)	−0.28
	Controls 2[d]	1.33 (1.18)	1.08 (0.52, 4.14)	(−0.74, 0.14)	1.43 (1.16)	1.13 (0.54, 3.81)	(−0.73, 0.18)
ΣPFHpS	Cases[c]	0.35 (0.18)	0.33 (0.15, 0.61)	−0.01	0.33 (0.18)	0.29 (0.12, 0.58)	−0.02
	Controls 2[d]	0.36 (0.18)	0.34 (0.17, 0.61)	(−0.09, 0.06)	0.35 (0.18)	0.34 (0.13, 0.61)	(−0.09, 0.05)

(*continued on next page*)

Table 1 (*continued*)

		T1 (pre-diagnosis)			T2 (post-diagnosis)		
		Mean (SD)	Median (5, 95 percentiles)	ΔMean$_{case-control}$ (95% CI)[e]	Mean (SD)	Median (5, 95 percentiles)	ΔMean$_{case-control}$ (95% CI)[e]
ΣPFOS	Cases[c]	21.6 (10.1)	19.1 (10.3, 37.9)	−1.68	18.2 (9.97)	15.5 (6.54, 32.0)	−1.94
	Controls 2[d]	23.3 (12.5)	23.2 (8.65, 43.5)	(−6.27, 2.91)	20.1 (10.8)	18.6 (7.72, 34.1)	(−6.51, 2.63)

Abbreviations: PFAA, perfluoroalkyl acid; T1, time point 1 (2001/2); T2, time point 2 (2005/6); SD, standard deviation; CI: confidence interval; BMI, body mass index; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; ΣPFOS, sum perfluorooctane sulfonate; ΣPFHxS, sum perfluorohexane sulfonate; ΣPFHpS, sum perfluoroheptane sulfonate.

[a] Case group 1, n = 44.

[b] Control group 1 matched on age at T1, n = 44.

[c] Case group 2, n = 41.

[d] Control group 2 matched on age at T1 and BMI at T2, n = 41.

[e] Mean difference between matched case-control pairs and 95% CI around the mean.

Taken together, the results at T1 and T2, combined with observations from previous studies, suggest no clear role of PFAAs in T2DM pathogenesis or its progression in general populations with low background exposure. However, there are no previous studies from other populations with a similar study design to which we can compare our findings.

Our study also examined the mean longitudinal changes in demographics and PFAA concentrations between the two blood measurements (~4 years) within the same individuals and across the cases and control groups. From T1 to T2, cases maintained a stable weight, whereas both control groups increased in mean body weight. This could be attributed to lifestyle changes adopted by cases after being diagnosed with T2DM. When comparing PFAA concentrations within the cases, PFOA and ΣPFOS concentrations significantly decreased from T1 to T2, whereas PFNA, PFDA, PFUnDA, and ΣPFHxS concentrations increased significantly. Similar to the trends in cases, ΣPFOS decreased significantly, and PFNA, PFDA, and PFUnDA concentrations increased significantly from T1 to T2 in both control groups. However, there were no significant changes in PFOA, ΣPFHxS, or ΣPFHpS concentrations. These findings are in line with previous studies of time trends conducted during the same time period that also included repeated measurements of PFAAs within the same individuals (Nøst et al., 2014; Olsen et al., 2007b, 2012; Toms et al., 2014). These studies reported declining PFOA and PFOS concentrations and increasing PFNA, PFDA, and PFUnDA concentrations (Fitz-Simon et al., 2013; Nøst et al., 2014; Olsen et al., 2007b, 2012; Toms et al., 2014). Thus, our study followed the temporal trends that could be expected for most PFAAs based on studies in other populations. The mean PFAA concentrations at both time points and the decline in PFOA and PFOS concentrations from T1 to T2 in our study were considerably lower than those in the studies mentioned above. One of the contributing factors could be the demographics of our study sample, which mainly included older women, among whom the decline in PFAA concentrations may be slower when compared to the younger women surveyed in previous studies.

When comparing longitudinal changes in PFAAs across cases and controls, no significant differences were observed for any of the PFAA compounds between the groups after controlling for confounding factors. Thus, cases and controls decreased or increased in a similar manner, which suggests that T2DM diagnosis, use of T2DM medication, and weight changes or BMI following T2DM diagnosis in cases have limited influence on PFAA concentrations. The longitudinal changes in PFAAs were rather influenced by age and changes in diet. No other prospective studies have been conducted among T2DM cases and controls with pre- and post-diagnostic PFAA measurements with which to compare the temporal changes observed in our study, suggesting the need for further longitudinal studies to explore intra-individual and inter-individual temporal changes in PFAAs between T2DM cases and controls, especially among individuals exposed to higher concentrations which would extend our understanding of how metabolic changes are related to PFAA concentrations.

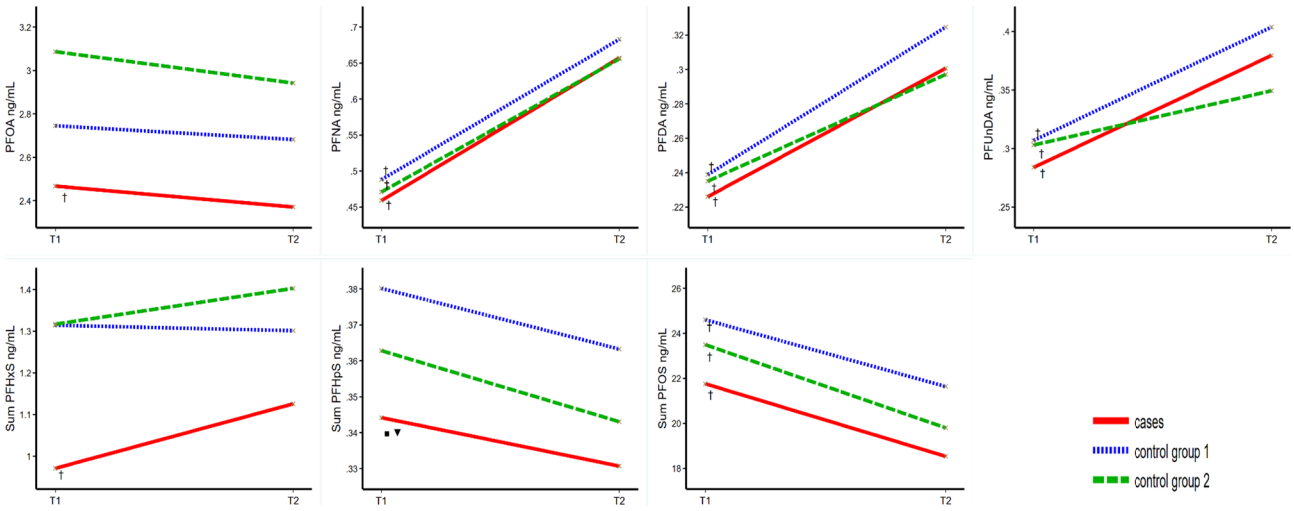

Fig. 2. Crude longitudinal changes (δ) in mean PFAA concentrations from T1 (2001/02, pre-diagnosis) to T2 (2005/06, post-diagnosis) in cases and control groups. †denotes significant change (paired *t*-test, $p < 0.05$) between T1 and T2 within the group; ■ denotes significant (one sample *t*-test, $p < 0.05$) difference in longitudinal change between cases and control group 1; ▼ denotes significant (one sample *t*-test, $p < 0.05$) difference in longitudinal change between cases and control group 2. Abbreviations: PFAA, perfluoroalkyl acid; T1, time point 1; T2, time point 2 PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate; PFHpS perfluoroheptane sulfonate.

Table 2
Odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between a one-interquartile range increase in perfluoroalkyl acid concentrations, and 50 ranks increase for the sum of PFAAs compounds and type 2 diabetes mellitus in case-control group 1 (matched by age, n = 44).

	T1 (pre-diagnosis)		T2 (post-diagnosis)	
PFAA compounds(ng/mL)	Age adjusted OR (95% CI)	Multivariable adjusted OR (95% CI)	Age adjusted OR (95% CI)	Multivariable adjusted OR (95% CI)
PFOA	0.73 (0.43, 1.28)	0.65 (0.34, 1.26)	0.74 (0.44, 1.25)	0.67 (0.36, 1.26)
PFNA	0.83 (0.52, 1.32)	0.80 (0.47, 1.36)	0.91 (0.53, 1.55)	0.80 (0.43, 1.48)
PFDA	0.88 (0.57, 1.38)	0.89 (0.55, 1.44)	0.85 (0.56, 1.30)	0.85 (0.53, 1.36)
PFUnDA	0.82 (0.54, 1.23)	0.83 (0.54, 1.26)	0.88 (0.55, 1.39)	0.90 (0.54, 1.53)
ΣPFHxS	0.79 (0.56, 1.12)	0.72 (0.49, 1.05)	0.87 (0.63, 1.21)	0.80 (0.54, 1.20)
ΣPFHpS	0.88 (0.62, 1.24)	0.84 (0.57, 1.24)	0.87 (0.61, 1.25)	0.87 (0.56, 1.34)
ΣPFOS	0.88 (0.63, 1.22)	0.87 (0.60, 1.25)	0.84 (0.58, 1.20)	0.84 (0.55, 1.28)
ΣPFAAs	0.93 (0.79, 1.10)	0.91 (0.76, 1.10)	0.93 (0.79, 1.09)	0.89 (0.73, 1.08)

Abbreviations: T1, time 1 (2001/2); T2, time 2 (2005/6); PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; ΣPFHxS, sum perfluorohexane sulfonate; ΣPFHpS, sum perfluoroheptane sulfonate; ΣPFOS, sum perfluorooctane sulfonate ΣPFAAs, sum perfluoroalkyl acids. [a]The models were adjusted for age, breastfeeding, fish, meat, dairy, fruits and vegetables intake.

This study is the first longitudinal study to measure plasma PFAA concentrations at two different time points, once prior to and once following T2DM diagnosis within the same individuals. This enabled us to examine the association between PFAAs and T2DM both prospectively and cross-sectionally within the same individuals. Further, we were also able to observe longitudinal changes in the different PFAA compounds intra-individually and between T2DM cases and controls, which is novel. Rigorous laboratory quality control procedures represent an additional strength of the present study. Potential confounders were either negated by matching for age, BMI, and year of blood collection, or adjusted for in the analyses. We used two different control groups: one matched on age and the other on age and BMI. Studies have shown that that elevated PFAAs concentrations are associated with weight gain/re-gain in obese individuals/individuals at high risk for T2DM undergoing a health intervention program (Cardenas et al., 2018; Liu et al., 2018). This may suggest that the results in our study show the indirect effect of PFAAs on T2DM masked by the matching on BMI for case-control group 2. However, previous studies from general populations (Barry et al., 2014; Blake et al., 2018; Lin et al., 2009; Nelson et al., 2010) have reported that PFAAs concentrations are not associated to BMI and our overall results also show that the ORs were similar for both case-control groups in the pre- and post-diagnostic associations. Therefore, we find it unlikely that this is a large bias in our study. Although, we do agree that matching by BMI is unnecessary in future studies. However, the potential for chance findings and bias from residual and unmeasured confounding is still possible. The conditional logistic regression models were adjusted for the selection bias introduced by the matching. However, this study is based on a small sample size, which limited the possibility to detect weak associations. The T2DM cases were chosen based on self-reported T2DM from questionnaires and were not confirmed by blood tests, as these blood samples were delivered several years before the present analyses. However, self-reported T2DM in the NOWAC study has been previously validated (Rylander et al., 2014). It should also be considered that the generalizability of this study may be limited to an older female Norwegian population.

This study adds to the evidence that neither pre- nor post-diagnostic measurements of PFAAs are associated with incident or prevalent T2DM, and that the temporal changes in PFAA concentrations are similar within T2DM cases and controls. Together, this suggests that cross-sectional studies of prevalent T2DM and PFAAs do not create biased results, as T2DM status, or post-diagnostic weight change merely influence longitudinal changes in PFAAs concentrations after diagnosis.

5. Conclusions

We observed no association between pre- or post-diagnostic PFAA concentrations and T2DM. The observed longitudinal changes in PFAA concentrations from pre- to post-T2DM diagnosis in cases were similar to the changes in controls.

Ethics approval and consent to participate

The NOWAC study has been approved by the Norwegian Data Inspectorate and the Regional Committee for Medical Research Ethics in Northern Norway. The present study was approved by the Regional Committee for Medical Research Ethics (REK, case number: 2015/1780). All participants provided written informed consent.

Table 3
Odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between a one-interquartile range increase in perfluoroalkyl acid concentrations, and 50 ranks increase for the sum of PFAAs compounds and type 2 diabetes mellitus in case-control group 2 (n = 41).

	T1 (pre-diagnosis)		T2 (post-diagnosis)	
PFAA compounds (ng/mL)	Age + BMI adjusted OR (95% CI)	Multivariable adjusted OR (95% CI)[a]	Age + BMI adjusted OR (95% CI)[a]	Multivariable adjusted OR (95% CI)[a]
PFOA	0.61 (0.35, 1.06)	0.73 (0.41, 1.32)	0.55 (0.31, 0.99)	0.57 (0.31, 1.04)
PFNA	0.94 (0.57, 1.57)	1.37 (0.69, 2.75)	0.97 (0.65, 1.43)	0.97 (0.64, 1.46)
PFDA	1.00 (0.62, 1.64)	1.52 (0.76, 3.07)	1.03 (0.70, 1.52)	1.03 (0.68, 1.54)
PFUnDA	0.93 (0.51, 1.70)	1.48 (0.64, 3.39)	1.21 (0.76, 1.92)	1.23 (0.75, 2.02)
ΣPFHxS	0.78 (0.53, 1.15)	0.70 (0.44, 1.09)	0.81 (0.58, 1.15)	0.76 (0.52, 1.11)
ΣPFHpS	0.92 (0.53, 1.59)	1.64 (0.71, 3.79)	0.87 (0.52, 1.44)	0.85 (0.50, 1.46)
ΣPFOS	0.81 (0.46, 1.42)	1.25 (0.57, 2.73)	0.79 (0.49, 1.28)	0.79 (0.48, 1.33)
ΣPFAAs	0.92 (0.79, 1.08)	1.00 (0.82, 1.24)	0.89 (0.75, 1.06)	0.88 (0.73, 1.06)

Abbreviations: T1, time 1 (2001/2); T2, time 2 (2005/6); PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; ΣPFHxS, sum perfluorohexane sulfonate; ΣPFHpS, sum perfluoroheptane sulfonate; ΣPFOS, sum perfluorooctane sulfonate ΣPFAAs, sum perfluoroalkyl acids. [a]The models were adjusted for age, BMI, breastfeeding, fish, meat, dairy, fruits and vegetables intake.

Funding

This work was supported by the Northern Norway Regional Authorities [project number: SFP1289] and Odd Bergs medical research fund (grant number: eph2017/254). The funders had no influence on the design of the study, in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

CRediT authorship contribution statement

Dolley Charles: Data curation, Formal analysis, Methodology, Visualization, Writing - original draft. **Vivian Berg:** Methodology, Visualization, Formal analysis, Validation, Writing - review & editing, Funding acquisition. **Therese H. Nøst:** Methodology, Visualization, Writing - review & editing. **Sandra Huber:** Formal analysis, Writing - review & editing. **Torkjel M. Sandanger:** Methodology, Visualization, Writing - review & editing. **Charlotta Rylander:** Conceptualization, Methodology, Supervision, Validation, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank the NOWAC study participants for their willingness to participate and donate blood, and Eiliv Lund for initiating, designing, and managing the NOWAC study for many years. We also wish to thank Bente A. Augdal for organizing and preparing the NOWAC blood sample collection, and the staff at the Department of Laboratory Medicine, University Hospital of North Norway, especially Tone F. Aune and Christina R. Hansen for assistance during sample preparation, analyses, and quantification work.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106095.

References

Alonso-Magdalena, P., Quesada, I., Nadal, A., 2011. Endocrine disruptors in the etiology of type 2 diabetes mellitus. Nat. Rev. Endocrinol. 7, 346–353. https://doi.org/10.1038/nrendo.2011.56.

Aune, D., Ursin, G., Veierod, M.B., 2009. Meat consumption and the risk of type 2 diabetes: A systematic review and meta-analysis of cohort studies. Diabetologia 52, 2277–2287. https://doi.org/10.1007/s00125-009-1481-x.

Barry, V., Darrow, L.A., Klein, M., Winquist, A., Steenland, K., 2014. Early life perfluorooctanoic acid (pfoa) exposure and overweight and obesity risk in adulthood in a community with elevated exposure. Environ. Res. 132, 62–69. https://doi.org/10.1016/j.envres.2014.03.025.

Bellou, V., Belbasis, L., Tzoulaki, I., Evangelou, E., 2018. Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. PLoS One 13, e0194127. https://doi.org/10.1371/journal.pone.0194127.

Blake, B.E., Pinney, S.M., Hines, E.P., Fenton, S.E., Ferguson, K.K., 2018. Associations between longitudinal serum perfluoroalkyl substance (pfas) levels and measures of thyroid hormone, kidney function, and body mass index in the fernald community cohort. Environ. Pollut. 242, 894–904. https://doi.org/10.1016/j.envpol.2018.07.042.

Cardenas, A., Gold, D.R., Hauser, R., Kleinman, K.P., Hivert, M.F., Calafat, A.M., et al., 2017. Plasma concentrations of per- and polyfluoroalkyl substances at baseline and associations with glycemic indicators and diabetes incidence among high-risk adults in the diabetes prevention program trial. Environ. Health. Perspect. 125, 107001. https://doi.org/10.1289/EHP1612.

Cardenas, A., Hauser, R., Gold, D.R., Kleinman, K.P., Hivert, M.F., Fleisch, A.F., et al., 2018. Association of perfluoroalkyl and polyfluoroalkyl substances with adiposity. JAMA. Netw. Open. 1, e181493. https://doi.org/10.2337/dc18-2254.

Christensen, K.Y., Raymond, M., Thompson, B.A., Anderson, H.A., 2016. Perfluoroalkyl substances in older male anglers in Wisconsin. Environ. Int. 91, 312–318. https://doi.org/10.1016/j.envint.2016.03.012.

Donat-Vargas, C., Bergdahl, I.A., Tornevi, A., Wennberg, M., Sommar, J., Kiviranta, H., et al., 2019. Perfluoroalkyl substances and risk of type 2 diabetes: A prospective nested case-control study. Environ. Int. 123, 390–398. https://doi.org/10.1016/j.envint.2018.12.026.

Fitz-Simon, N., Fletcher, T., Luster, M.I., Steenland, K., Calafat, A.M., Kato, K., et al., 2013. Reductions in serum lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid. Epidemiology 24, 569–576. https://doi.org/10.1097/EDE.0b013e31829443ee.

Hatch, E.E., Nelson, J.W., Stahlhut, R.W., Webster, T.F., 2010. Association of endocrine disruptors and obesity: Perspectives from epidemiological studies. Int. J. Androl. 33, 324–332. https://doi.org/10.1111/j.1365-2605.2009.01035.x.

Haug, L.S., Huber, S., Becher, G., Thomsen, C., 2011. Characterisation of human exposure pathways to perfluorinated compounds–comparing exposure estimates with biomarkers of exposure. Environ. Int. 37, 687–693. https://doi.org/10.1016/j.envint.2011.01.011.

He, X., Liu, Y., Xu, B., Gu, L., Tang, W., 2018. PFOA is associated with diabetes and metabolic alteration in us men: National health and nutrition examination survey 2003–2012. Sci. Total. Environ. 625, 566–574. https://doi.org/10.1016/j.scitotenv.2017.12.186.

Huber, S., Brox, J., 2015. An automated high-throughput SPE micro-elution method for perfluoroalkyl substances in human serum. Anal. Bioanal. Chem. 407, 3751–3761. https://doi.org/10.1007/s00216-015-8601-x.

Imamura, F., O'Connor, L., Ye, Z., Mursu, J., Hayashino, Y., Bhupathiraju, S.N., et al., 2015. Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: Systematic review, meta-analysis, and estimation of population attributable fraction. BMJ 351, h3576. https://doi.org/10.1136/bmj.h3576.

Jiang, Q., Gao, H., Zhang, L., 2015. Metabolic effects PFAS. In: DeWitt, J.C. (Ed.), Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. Springer International Publishing, pp. 177–201. https://doi.org/10.1007/978-3-319-15518-0_7.

Karnes, C., Winquist, A., Steenland, K., 2014. Incidence of type 2 diabetes in a cohort with substantial exposure to perfluorooctanoic acid. Environ. Res. 128, 78–83. https://doi.org/10.1016/j.envres.2013.11.003.

Khalil, N., Lee, M., Steenland, K., 2015. Epidemiological findings. In: DeWitt, J.C. (Ed.), Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. Springer International Publishing, pp. 305–335. https://doi.org/10.1007/978-3-319-15518-0_13.

Lau, C., 2015. Perfluorinated compounds: An overview. In: DeWitt, J.C. (Ed.), Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. Springer International Publishing, pp. 1–21. https://doi.org/10.1007/978-3-319-15518-0_1.

Lin, C.Y., Chen, P.C., Lin, Y.C., Lin, L.Y., 2009. Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults. Diabetes Care 32, 702–707. https://doi.org/10.2337/dc08-1816.

Lin Pi, I.D., Cardenas, A., Hauser, R., Gold, D.R., Kleinman, K.P., Hivert, M.F., et al., 2020. Dietary characteristics associated with plasma concentrations of per- and polyfluoroalkyl substances among adults with pre-diabetes: Cross-sectional results from the diabetes prevention program trial. Environ. Int. 137, 105217. https://doi.org/10.1016/j.envint.2019.105217.

Lind, L., Zethelius, B., Salihovic, S., van Bavel, B., Lind, P.M., 2014. Circulating levels of perfluoroalkyl substances and prevalent diabetes in the elderly. Diabetologia 57, 473–479. https://doi.org/10.1007/s00125-013-3126-3.

Lind, P.M., Lind, L., 2018. Endocrine-disrupting chemicals and risk of diabetes: An evidence-based review. Diabetologia 61, 1495–1502. https://doi.org/10.1007/s00125-018-4621-3.

Liu, G., Dhana, K., Furtado, J.D., Rood, J., Zong, G., Liang, L., et al., 2018. Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: A prospective study. PLoS Med. 15, e1002502. https://doi.org/10.1371/journal.pmed.1002502.

Lund, E., Dumeaux, V., Braaten, T., Hjartaker, A., Engeset, D., Skeie, G., et al., 2008. Cohort profile: The norwegian women and cancer study–NOWAC–kvinner og kreft. Int. J. Epidemiol. 37, 36–41. https://doi.org/10.1093/ije/dym137.

MacNeil, J., Steenland, N.K., Shankar, A., Ducatman, A., 2009. A cross-sectional analysis of type 2 diabetes in a community with exposure to perfluorooctanoic acid (PFOA). Environ. Res. 109, 997–1003. https://doi.org/10.1016/j.envres.2009.08.002.

Magliano, D.J., Loh, V.H.Y., Harding, J.L., Botton, J., Shaw, J.E., 2014. Persistent organic pollutants and diabetes: A review of the epidemiological evidence. Diabetes Metab. 40, 1–14. https://doi.org/10.1016/j.diabet.2013.09.006.

McAllister, E.J., Dhurandhar, N.V., Keith, S.W., Aronne, L.J., Barger, J., Baskin, M., et al., 2009. Ten putative contributors to the obesity epidemic. Crit. Rev. Food. Sci. Nutr. 49, 868–913. https://doi.org/10.1080/10408390903372599.

Nadal, A., Quesada, I., Tuduri, E., Nogueiras, R., Alonso-Magdalena, P., 2017. Endocrine-disrupting chemicals and the regulation of energy balance. Nat. Rev. Endocrinol. 13, 536–546. https://doi.org/10.1038/nrendo.2017.51.

Nelson, J.W., Hatch, E.E., Webster, T.F., 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ. Health. Perspect. 118, 197–202. https://doi.org/10.1289/ehp.0901165.

Nøst, T.H., Vestergren, R., Berg, V., Nieboer, E., Odland, J.O., Sandanger, T.M., 2014. Repeated measurements of per- and polyfluoroalkyl substances (PFASs) from 1979 to 2007 in males from northern Norway: Assessing time trends, compound correlations and relations to age/birth cohort. Environ. Int. 67, 43–53. https://doi.org/10.1016/j.envint.2014.02.011.

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L.,

et al., 2007a. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health. Perspect. 115, 1298–1305. https://doi.org/10.1289/ehp.10009.

Olsen, G.W., Mair, D.C., Reagen, W.K., Ellefson, M.E., Ehresman, D.J., Butenhoff, J.L., et al., 2007b. Preliminary evidence of a decline in perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations in american red cross blood donors. Chemosphere 68, 105–111. https://doi.org/10.1016/j.chemosphere.2006.12.031.

Olsen, G.W., Lange, C.C., Ellefson, M.E., Mair, D.C., Church, T.R., Goldberg, C.L., et al., 2012. Temporal trends of perfluoroalkyl concentrations in american red cross adult blood donors, 2000–2010. Environ. Sci. Technol. 46, 6330–6338. https://doi.org/10.1021/es300604p.

Rylander, C., Sandanger, T.M., Engeset, D., Lund, E., 2014. Consumption of lean fish reduces the risk of type 2 diabetes mellitus: A prospective population based cohort study of norwegian women. PLoS. One. 9, e89845. https://doi.org/10.1371/journal.pone.0089845.

Rylander, C., Sandanger, T.M., Nost, T.H., Breivik, K., Lund, E., 2015. Combining plasma measurements and mechanistic modeling to explore the effect of POPs on type 2 diabetes mellitus in norwegian women. Environ. Res. 142, 365–373. https://doi.org/10.1016/j.envres.2015.07.002.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., et al., 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes atlas, 9(th) edition. Diabetes Res. Clin. Pract. 157, 107843. https://doi.org/10.1016/j.diabres.2019.107843.

Sun, Q., Zong, G., Valvi, D., Nielsen, F., Coull, B., Grandjean, P., 2018. Plasma concentrations of perfluoroalkyl substances and risk of type 2 diabetes: A prospective investigation among U.S. Women. Environ. Health. Perspect. 126, 037001. https://doi.org/10.1289/EHP2619.

Taylor, K.W., Novak, R.F., Anderson, H.A., Birnbaum, L.S., Blystone, C., Devito, M., et al., 2013. Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: A national toxicology program workshop review. Environ. Health. Perspect. 121, 774–783. https://doi.org/10.1289/ehp.1205502.

Toms, L.M., Thompson, J., Rotander, A., Hobson, P., Calafat, A.M., Kato, K., et al., 2014. Decline in perfluorooctane sulfonate and perfluorooctanoate serum concentrations in an Australian population from 2002 to 2011. Environ. Int. 71, 74–80. https://doi.org/10.1016/j.envint.2014.05.019.

Tornevi, A., Sommar, J., Rantakokko, P., Akesson, A., Donat-Vargas, C., Kiviranta, H., et al., 2019. Chlorinated persistent organic pollutants and type 2 diabetes – a population-based study with pre- and post- diagnostic plasma samples. Environ. Res. 174, 35–45. https://doi.org/10.1016/j.envres.2019.04.017.

Vestergren, R., Cousins, I.T., Trude, l.D., Wormuth, M., Scheringer, M., 2008. Estimating the contribution of precursor compounds in consumer exposure to pfos and pfoa. Chemosphere 73, 1617–1624. https://doi.org/10.1158/1055-9965.EPI-10-0992.

Vestergren, R., Cousins, I.T., 2009. Tracking the pathways of human exposure to perfluorocarboxylates. Environ. Sci. Technol. 43, 5565–5575. https://doi.org/10.1021/es900228k.

Waaseth, M., Bakken, K., Dumeaux, V., Olsen, K.S., Rylande, C., Figenschau, Y., et al., 2008. Hormone replacement therapy use and plasma levels of sex hormones in the norwegian women and cancer postgenome cohort - a cross-sectional analysis. BMC Womens Health 8, 1. https://doi.org/10.1186/1472-6874-8-1.

Wolf, C.J., Takacs, M.L., Schmid, J.E., Lau, C., Abbott, B.D., 2008. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol. Sci. 106, 162–171. https://doi.org/10.1093/toxsci/kfn166.